

**EXHIBIT B**

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### **Sample Articles**

- 1. Differential effects of bone graft substitutes on regeneration of bone marrow** Schwartz Z, Doukarsky-Marx I, Nasatzky E, Goultchin J, Ranly DM, Greenspan DC, Sela J, Boyan BD. *Clin Oral Implants Res*. 2008 Dec;19(12):1233-45.

**Background:** This study used a rat tibial marrow ablation model to test the hypothesis that bone remodeling within the medullary canal varies with bone graft materials of different chemical compositions and structural properties, impacting marrow cavity restoration. Bone graft materials were selected based on their relative resorption or degradation in vivo and their osteogenic properties. **Methods:** Following ablation of the right tibial marrow in male Sabra-strain rats, materials were implanted in the proximal marrow cavity: poly-d,L-lactide-co-glycolide 75 : 25 (PLGA); coralline-hydroxyapatite (HA); calcium-sulfate (CaSO4); collagen-HA-tricalcium phosphate granules; anorganic bovine bone mineral; demineralized bone matrix (DBM); 45S5 Bioglass (BG); PLGA with BG 50 : 50; PLGA : BG 80 : 20; and PLGA and PLGA:BG 50 : 50 plus bone marrow (BM). Control tibias were ablated but received no implants. At 2 (endosteal bone healing), 4 (marrow cavity remodeling) and 8 weeks (marrow restoration), six to eight animals per group were euthanized and tibias processed for histomorphometry of proximal and distal medullary canals. **Results:** Control tibias showed primary bone in proximal and distal medullary canals at 2 weeks, with trabeculae surrounded by cellular marrow. At 4 and 8 weeks, control trabeculae were thinned and marrow had more fat cells. In the treated tibias, trabecular bone volume (TBV) varied with time and was material specific. Most implants supported comparable TBV at 2 weeks. Sites with CaSO4 or DBM exhibited decreased TBV with time whereas trabecular bone was retained in proximal tibias containing other materials, closely juxtaposed to the implants. TBV did not always correlate directly with implant volume, but changes in BM volume were correlated inversely with TBV. Addition of BM increased marrow restoration in sites containing PLGA; however, BM reduced restoration of marrow when added to PLGA : BG. Although the presence of implants in the proximal tibia resulted in retention of trabecular bone, there was a time-dependent reduction in TBV in distal canals; the rate and extent of the distal TBV reduction were implant dependent. **Conclusions:** Thus, although many materials can support bone formation in the marrow cavity, bone quality, quantity, and physical relationship to the implant, and its rate of resorption differ in a material-dependent manner, resulting in differences in the restoration of marrow. **Clinical relevance:** Bone graft materials should be selected not only for their ability to support new bone formation but also for their impact on the remodeling phase of bone healing.

- 2. Development of a synthetic bone scaffold using porous hydroxyapatite for bone repair** Mustaffa R, Besar I, Andanastuti M, *Med J Malaysia*. 2008 Jul;63 Suppl A:95-6

In this study, porous hydroxyapatite (HA) samples were fabricated via sponge techniques with the aid of sago as part of the binder mixture. Development processes for the production of porous bone graft substitutes are studied using polyurethane sponge. To obtain the optimum amount of binder for successful fabrication of porous HA were done. Initially, porous HA powder was synthesized using calcium hydroxide and orthophosphoric acid. Meanwhile, sago was mixed with PVA in a certain ratio to be used as binder for preparing the porous HA. After a series of investigative tests were conducted to characterize the sintered samples, the use of the sago and polymeric mixture was found to successfully aid the fabrication of porous HA samples. In this investigation, comparison of physical and mechanical characteristics between samples prepared using difference techniques was made.

- 3. Advances in craniofacial surgery** Tatum SA, Losquadro WD. *Arch Facial Plast Surg*. 2008 Nov-Dec;10(6):376-80

The past 10 years have witnessed many advances in craniofacial surgery. Advances in surgical techniques, such as distraction osteogenesis and endoscopic procedures, combined with refinements in surgical equipment, such as resorbable plating and distractors, have improved surgical outcomes, while minimizing morbidity. Technological advances in 3-dimensional imaging, computer simulation, and intraoperative navigation facilitate diagnosis, preoperative planning, and surgical execution. Rising cases of deformational plagiocephaly owing to increased supine infant sleep positioning necessitated the development of appropriate diagnosis and treatment and the avoidance of unnecessary surgery. A greater understanding of the genetic basis of craniofacial disorders has allowed better preoperative assessment and counseling. Finally, efforts to develop better bone graft substitutes with gene therapy and nanotechnology are ongoing.

**4. ISSLS Prize Winner: Positron Emission Tomography and Magnetic Resonance Imaging for Monitoring Interbody Fusion With Equine Bone Protein Extract, Recombinant Human Bone Morphogenetic Protein-2, and Autograft.**

Foldager C, Bendtsen M, Zou X, Zou L, Olsen AK, Munk OL, Stødkilde-Jørgensen H, Bünger C. *Spine.* 2008 Nov 11

**STUDY DESIGN.:** Prospective and randomized experimental study with anterior lumbar interbody fusion in a porcine model. **OBJECTIVE.:** To assess the early time-course of spinal fusion with equine bone protein extract (COLLOSS E), recombinant human bone morphogenetic protein-2 (rhBMP-2), and autograft using quantitative methods of positron emission tomography (PET)/computed tomography and magnetic resonance imaging (MRI). **SUMMARY OF BACKGROUND DATA.:** Different growth and differentiation factors are currently being used for inducing bone formation in spinal fusion. However, the mechanisms and time-course of bone formation using these graft substitutes remain less known. **METHODS.:** Eighteen female Danish landrace pigs underwent a 3-level anterior lumbar interbody fusion procedure from L3-L6. A PEEK cage, packed with COLLOSS E, rhBMP-2, or autograft, was randomly placed. Each group of 6 pigs was observed for 2, 4, or 8 weeks, respectively. F PET/computed tomography and MRI examinations were performed, and data were correlated with histomorphometry. PET data were analyzed using a Gjedde-Patlak plot. K-values from the plot correspond to the metabolic rate. T2-values were calculated by T2 mapping. **RESULTS.:** rhBMP-2 presented the highest bone formation on histologic sections at 25.6% at 4 weeks after surgery. Eight weeks after surgery, autograft had the highest bone formation with 37.3%, which was significantly higher than rhBMP-2 at 30.5% ( $P < 0.05$ ), and higher than COLLOSS E at 27.0% ( $P = 0.06$ ). COLLOSS E and rhBMP-2 had significantly higher K-values than autograft ( $P < 0.05$ ) at 2 weeks after surgery. There were no differences in K-values between COLLOSS E and autograft at 4 and 8 weeks. However, rhBMP-2 was significantly higher at 4 weeks and lower at 8 weeks than these 2 ( $P < 0.05$ ). Linear correlation,  $R = 0.8275$ , was observed for intertrabecular volume/total volume and T2-values. **CONCLUSION.:** PET and MRI are valid tools for monitoring the process of interbody fusion *in vivo*. Osteogenic mechanisms using COLLOSS E resembles that of autograft by the process of endochondral ossification. rhBMP-2 deposits osteoid directly on the collagen network.

**5. Preservation of the postextraction alveolar ridge: a clinical and histologic study** Cardaropoli D, Cardaropoli G *Int J Periodontics Restorative Dent.* 2008 Oct;28(5):469-77

The aim of this investigation was to assess the possibility of preserving the buccal and lingual plates of a postextraction socket from resorption using bone filler after tooth extraction. In 10 patients, 10 single extraction sites in the posterior area received a bone substitute. The osteoconductive material was covered by a collagen membrane in all cases. Contour changes of the alveolar process were evaluated intraorally using a surgical caliper over a 4-month period. Four months after extraction, a specimen was harvested from the area previously augmented with bone filler, and histologic analysis was performed. The results demonstrated that it was possible to preserve about 85% of the initial ridge dimensions, allowing for correct implant placement. From a histologic point of view, new bone

formation was detected in all sites, with a 25% average residual presence of the graft particles. This investigation confirms the benefit of augmenting an extraction socket with bone substitutes.

6. **Bioactive glass and autogenous bone as bone graft substitutes in benign bone tumors.** Lindfors NC, Heikkilä JT, Koski I, Mattila K, Aho AJ. *Biomed Mater Res B Appl Biomater.* 2008 Nov 5

In a prospective randomized study, 25 patients with benign bone tumors were surgically treated with either bioactive glass S53P4 (BG) or autogenous bone (AB) as bone graft material. X-rays were taken preoperatively and postoperatively at 2 weeks and at 3, 8, 12, 18, 24, and 36 months. In addition, for most of the patients, CT scans were performed at the same time-points. No infections or material-related adverse reactions occurred in any patient. The filled cavity was replaced faster by new bone in the AB group than in the BG group ( $p = 0.0001$ ). However, at 36 months, no statistical difference in cavity volume between the two groups was observed on X-rays ( $p = 0.7881$ ) or on CT scans ( $p = 0.9117$ ). In the BG group at 3 years, the filled cavity appeared, however, dense on X-rays, and glass granules on CT scans were observed. During the follow-up period, the cortical thickness seemed to increase more in the BG group than in the AB group ( $p < 0.0001$ ). (c) 2008 Wiley Periodicals, Inc. *J Biomed Mater Res Part B: Appl Biomater* 2009.

7. **SHED repair critical-size calvarial defects in mice** Seo BM, Sonoyama W, Yamaza T, Coppe C, Kikuchi T, Akiyama K, Lee JS, Shi S *Oral Dis.* 2008 Jul;14(5):428-34

OBJECTIVE: Stem cells from human exfoliated deciduous teeth (SHED) are a population of highly proliferative postnatal stem cells capable of differentiating into odontoblasts, adipocytes, neural cells, and osteo-inductive cells. To examine whether SHED-mediated bone regeneration can be utilized for therapeutic purposes, we used SHED to repair critical-size calvarial defects in immunocompromised mice. MATERIALS AND METHODS: We generated calvarial defects and transplanted SHED with hydroxyapatite/tricalcium phosphate as a carrier into the defect areas. RESULTS: SHED were able to repair the defects with substantial bone formation. Interestingly, SHED-mediated osteogenesis failed to recruit hematopoietic marrow elements that are commonly seen in bone marrow mesenchymal stem cell-generated bone. Furthermore, SHED were found to co-express mesenchymal stem cell marker, CC9/MUC18/CD146, with an array of growth factor receptors such as transforming growth factor beta receptor I and II, fibroblast growth factor receptor I and III, and vascular endothelial growth factor receptor I, implying their comprehensive differentiation potential. CONCLUSIONS: Our data indicate that SHED, derived from neural crest cells, may select unique mechanisms to exert osteogenesis. SHED might be a suitable resource for orofacial bone regeneration.

8. **Preliminary investigation of novel bone graft substitutes based on strontium-calcium-zinc-silicate glasses** Boyd D, Carroll G, Towler MR, Freeman C, Farthing P, Brook IM *J Mater Sci Mater Med.* 2008 Oct 7

Bone graft procedures typically require surgeons to harvest bone from a second site on a given patient (Autograft) before repairing a bone defect. However, this results in increased surgical time, excessive blood loss and a significant increase in pain. In this context a synthetic bone graft with excellent histocompatibility, built in antibacterial efficacy and the ability to regenerate healthy tissue in place of diseased tissue would be a significant step forward relative to current state of the art philosophies. We developed a range of calcium-strontium-zinc-silicate glass based bone grafts and characterised their structure and physical properties, then evaluated their in vitro cytotoxicity and in vivo biocompatibility using standardised models from the literature. A graft (designated BT109) of composition 0.28SrO/0.32ZnO/0.40 SiO<sub>2</sub> (mol fraction) was the best performing formulation in vitro shown to induce extremely mild cytopathic effects (cell viability up to 95%) in comparison with the commercially available bone graft Novabone(R) (cell viability of up to 72%). Supplementary to this, the grafts were examined using the standard rat femur healing model on healthy Wister rats. All grafts were shown to be equally well tolerated in bone tissue and new bone was seen in close apposition to implanted particles with no evidence of an inflammatory response within bone. Complimentary to this BT109 was implanted into the femurs of

ovariectomized rats to monitor the response of osteoporotic tissue to the bone grafts. The results from this experiment indicate that the novel grafts perform equally well in osteoporotic tissue as in healthy tissue, which is encouraging given that bone response to implants is usually diminished in ovariectomized rats. In conclusion these materials exhibit significant potential as synthetic bone grafts to warrant further investigation and optimization

9. **Application of BMP-7 to tibial non-unions: a 3-year multicenter experience** Kanakaris NK, Calori GM, Verdonk R, Burssens P, De Biase P, Capanna R, Vangosa LB, Cherubino P, Baldo F, Ristiniemi J, Kontakis G, Giannoudis PV. *Injury*. 2008 Sep;39 Suppl 2:S83-90

**SUMMARY:** The effective treatment of the often debilitating, longlasting and large-asset-consuming complication of fracture non-unions has been in the centre of scientific interest the last decades. The use of alternative bone substitutes to the gold standard of autologous graft includes the osteoinductive molecules named bone morphogenetic proteins (BMPs). A multicenter registry and database (bmpusergroup.co.uk) focused on the application of BMP-7/OP-1 was created in December 2005. We present the preliminary results, using the prospective case-series of aseptic tibial non-unions as an example. Sixty-eight patients fulfilled the inclusion criteria for this observational study, with a minimum follow-up of 12 months. The median duration of tibial non-union prior to BMP-7 application was 23 months (range 9-317 mo). Patients had undergone a median of 2 (range 0-11) revision procedures prior to the administration of BMP-7. In 41% the application of BMP-7 was combined with revision of the fixation at the non-union site. Non-union healing was verified in 61 (89.7%) in a median period of 6.5 months (range 3-15 mo). No adverse events or complications were associated with BMP-7 application. The safety and efficacy of BMP-7 was verified in our case series, and was comparable to the existing evidence. The establishment of multicenter networks and the systematic and long-term follow- up of these patients are expected to provide further information and significantly improve our understanding of this promising osteoinductive bone substitute.

10. **Comparison of Biomaterial Implants in the Dental Socket: Histological Analysis in Dogs.** Santos FA, Pochapski MT, Martins MC, Zenóbio EG, Spolidoro LC, Marcantonio E Jr. *Clin Implant Dent Relat Res*. 2008 Sep 9.

**Background:** Bone graft procedures have been used commonly in buco-maxillo-facial surgery. For this reason, many researchers have evaluated the bone substitutes. **Purpose:** The present study evaluated soft and hard tissue reactions to two different hydroxyapatites HAs (synthetic HA and natural HA) and bioactive glass implanted into the sockets immediately after extraction. **Materials and Methods:** First and third upper and lower premolars, on both sides, were extracted from six female dogs. The alveolar sockets were randomly assigned to four groups: Group 1 - control (unfilled), Group 2 - filled with synthetic hydroxyapatite, Group 3 - filled with bovine bone mineral (natural HA), and Group 4 - filled with bioactive glass. The animals were euthanized at 4 weeks (n = 2), 8 weeks (n = 2), and 28 weeks (n = 2) after extraction. The mandible and maxilla of each animal were removed for histological analysis to determine soft tissue reactions, newly formed bone, bone characteristics, and presence or absence of implanted materials. **Results:** Most particles of synthetic hydroxyapatite had bone formation on their surface, although some particles showed a layer of fibrous connective tissue. The bovine bone mineral group exhibited particles partially replaced with bone formation. The bioactive glass group showed particles with a thin layer of calcified tissue, but was absent in some specimens, suggesting complete resorption. **Conclusion:** All biomaterials had similar behavior. Bovine bone mineral, compared to synthetic hydroxyapatite and bioactive glass, showed a larger number of particles covered with osseous tissue. All biomaterials interfered with the socket repair process.

11. **Long-term clinical results on the use of bone-replacement grafts in the treatment of intrabony periodontal defects. Comparison of the use of autogenous bone graft plus calcium sulfate to autogenous bone graft covered**

**with a bioabsorbable membrane.** Orsini M, Orsini G, Benloch D, Aranda JJ, Sanz M. J Periodontol. 2008 Sep;79(9):1630-7

**BACKGROUND:** Many clinical investigations have demonstrated the benefits of different regenerative approaches in the treatment of infrabony defects. The aim of this investigation was to evaluate the results obtained with the combination of autogenous bone grafting plus calcium sulfate and to compare this outcome with the results obtained using autogenous bone grafting with a bioabsorbable membrane. **METHODS:** Twelve subjects participated in this split-mouth trial. Twelve 2- or 3-wall periodontal defects were treated with a combination of autogenous bone grafting plus calcium sulfate (test) and were compared to 12 defects treated with autogenous bone grafting with a bioabsorbable membrane (control). Before surgery, subjects were instructed in oral hygiene techniques, and scaling and root planing were completed. Surgery was identical for both groups except for the regenerative material. After healing, subjects followed a periodontal maintenance program. Probing depth (PD), clinical attachment level, and bleeding on probing were recorded at baseline, 6 months, and 6 years. **RESULTS:** There were no statistical differences between test and control defects at baseline. At 6 months, there was a PD reduction of 4.3 +/- 1.0 mm in the control group and 4.4 +/- 1.1 mm in the test group. There was a clinical attachment gain of 3.5 +/- 1.1 mm in the control group and 3.6 +/- 1.0 mm in the test group. At 6 years, there was a PD reduction of 3.3 +/- 1.0 mm in the control group and 4.2 +/- 1.2 mm in the test group. There was a clinical attachment gain of 2.6 +/- 1.2 mm in the control group and 2.4 +/- 1.1 mm in the test group. Differences between treatment groups were not statistically significant at 6 months or 6 years. **CONCLUSION:** Both therapies led to significant short- and long-term improvements in the outcome variables assessed.

**12. Bioactivity of bone resorptive factor loaded on osteoconductive matrices: Stability post-dehydration.** Le Nihouannen D, Komarova SV, Gbureck U, Barralet JE. Eur J Pharm Biopharm. 2008 Aug 14

Since calcium phosphate cements were proposed two decades ago, extensive research has been realized to develop and improve their properties. They have proved their efficiency as bone graft substitutes and their ability to incorporate and release drugs. However, to date, all 'resorbable' osteoconductive synthetic biomaterials are in fact simply soluble. In order to investigate a synthetic material capable of inducing osteoclast remodelling post-implantation, a formulation of calcium phosphate cement loaded with a pro-resorptive cytokine (RANKL) was studied. Many prior release studies on calcium phosphates did not confirm that the matrix had no detrimental effect on the molecule to be released during storage prior to use or that bioactivity was maintained during storage. In this report, the stability of our protein was tested after loading onto the cement, and various regimens to improve stability were compared. The presence of trehalose was shown to stabilize the bioactivity of RANKL adsorbed to brushite cement. The reduction of both moisture and oxygen in the storage vessel improved osteoclastogenic potential of the matrix compared with that stored in ambient atmosphere and temperature. No loss in activity was observed over the study period for the loaded matrix stored in dry nitrogen.

**13. Comparison of osteogenic potential between apatite-coated poly(lactide-co-glycolide)/hydroxyapatite particulates and Bio-Oss.** Kim SS, Kim BS. Dent Mater J. 2008 May;27(3):368-75.

Previously, we developed a poly(lactide-co-glycolide)/nano-hydroxyapatite (PLGA/HA) composite that overcame the limitations of conventional ceramic bone substitutes. This was achieved by introducing a bone-like apatite layer on the composite to further enhance its osteogenic potential. In this study, we compared the osteogenic potential of the apatite-coated PLGA/HA particulates to that of Bio-Oss, a deproteinized bovine bone material. A mixture of fibrin gel and either apatite-coated PLGA/HA particulates or Bio-Oss was implanted into critical-size rat calvarial defects. As a control, fibrin gel was implanted alone into the defects. At eight weeks after treatment, histological examination showed new bone formation around the grafting materials, and bone formation was similar between the two groups. In the control group, bone was not regenerated and the defects were filled with

fibrous tissues. This study showed that a synthetic bone graft material, apatite-coated PLGA/HA particulates, had a comparable bone regeneration potential to the bovine-derived bone graft material, Bio-Oss.

14. **Surface modification of biodegradable polymer/TCP scaffolds and related research** Ma X, Hu Y, Wu X, Yan Y, Xiong Z, Lu R, Wang J, Li D, Xu X. Sheng Wu Yi Xue Gong Cheng Xue Za Zhi. 2008 Jun;25(3):571-7
- Under laboratory condition, the compound materials of Poly (DL-lactic-co-glycolic acid)/Tricalcium phosphate [PLGA/TCP(L), with component ratio of 7:3] were fabricated by combining the thermally induced phase separation (TIPS) with solvent-casting particulate-leaching (SCPL) approach. On the other hand, rapid prototyping (RP) technique manufactured PLGA/TCP scaffolds [PLGA/TCP(RP)] were obtained. These two kinds of carriers were coated with collagen type I (Col I). The extracted bovine bone morphogenetic protein (bBMP) was loaded into carriers to establish biomimetic synthetic bones. PLGA/TCP(L) scaffolds, demineralized bone matrices (DBM) of bovine cancellous bone, PLGA/TCP(L) scaffolds, biomimetic synthetic bones and OsteoSet bone graft substitutes were investigated. Scanning electron microscopy revealed that the microarchitecture of PLGA/TCP(RP) scaffolds was much better than that of PLGA/TCP(L) scaffolds. The diameter of macropore of PLGA/TCP(RP) scaffold was 350 microm. The porosities of PLGA/ TCP(L) scaffolds, DBM, PLGA/TCP(RP) scaffolds and OsteoSet bone graft substitutes were 21.5%, 70.4%, 58.6% and 0%, respectively (P<0.01). Modification of PLGA/TCP scaffolds with collagen type I [PLGA/TCP(L)-Col I and PLGA/TCP(RP)-Col I] essentially increased the affinity of the carriers to bBMP. Among these synthetic materials, PLGA/TCP(RP)-Col I-bBMP composite is promising as a novel bone graft substitute due to its advanced fabrication technique, good tri-dimensional microarchitecture and ideal components.
15. **Simple preservation of a maxillary extraction socket using beta-tricalcium phosphate with type I collagen: preliminary clinical and histomorphometric observations** Brkovic BM, Prasad HS, Konandreas G, Milan R, Antunovic D, Sándor GK, Rohrer MD. Brkovic BM, Prasad HS, Konandreas G, Milan R, Antunovic D, Sándor GK, Rohrer MD. J Can Dent Assoc. 2008 Jul-Aug;74(6):523-8
- Alveolar atrophy following tooth extraction remains a challenge for future dental implant placement. Immediate implant placement and postextraction alveolar preservation are 2 methods that are used to prevent significant postextraction bone loss. In this article, we report the management of a maxillary tooth extraction socket using an alveolar preservation technique involving placement of a cone of beta-tricalcium phosphate (beta -TCP) combined with type I collagen without the use of barrier membranes or flap surgery. Clinical examination revealed solid new bone formation 9 months after the procedure. At the time of implant placement, histomorphometric analysis of the biopsied bone showed that it contained 62.6% mineralized bone, 21.1% bone marrow and 16.3% residual beta -TCP graft. The healed bone was able to support subsequent dental implant placement and loading.